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Factors Which Affect the Amount of Inorganic Phosphate, Phosphorylcholine, and Phosphorylethanolamine in Xylem Exudate of Tomato Plants[1]

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ABSTRACT

Phosphate in the xylem exudate of tomato (*Lycopersicon esculentum*) plants was 70 to 98% inorganic phosphate (Pi), 2 to 30% P-choline, and less than 1% P-ethanolamine. Upon adding ^{32}Pi to the nutrient, Pi in xylem exudate had the same specific activity within 4 hours. P-choline and P-ethanolamine reached the same specific activity only after 96 hours. The amount of Pi in xylem exudate was dependent on Pi concentration in the nutrient and decreased from 1700 to 170 micromolar when Pi in the nutrient decreased from 50 to 2 micromolar. The flux of 0.4 nmoles organic phosphate per minute per gram fresh weight root into the xylem exudate was not affected by the Pi concentration in the nutrient solution unless it was below 1 micromolar. During 7 days of Pi starvation, Pi in the xylem exudate decreased from 1400 to 130 micromolar while concentrations of the two phosphate esters remained unchanged.

The concentration of phosphate esters in the xylem exudate was increased by addition of choline or ethanolamine to the nutrient solution, but Pi remained unchanged. Upon adding [^{14}C]choline to the nutrient, 10 times more [^{14}C]P-choline than [^{14}C]choline was in the xylem exudate and 85 to 90% of the ester phosphate was P-choline. When [^{14}C]ethanolamine was added, [^{14}C]P-ethanolamine and [^{14}C]ethanolamine in the xylem sap were equal in amount. P-choline and P-ethanolamine accumulated in leaves of whole plants at the same time and the same proportion as observed for their flux into the xylem exudate. No relationship between the transport of P-choline and Pi in the xylem was established. Rather, the amount of choline in xylem exudate and its incorporation into phosphatidylcholine in the leaf suggest that the root is a site of synthesis of P-choline and P-ethanolamine for phospholipid synthesis in tomato leaves.

In 1933, organic phosphate was estimated to comprise approximately 32% of the total phosphate exuded by field-grown corn plants (14). Chromatographic investigations in 1956 (10, 18) revealed that Pi and two organic P-esters were labeled in xylem exudates of tomato, barley, and bean plants after ^{32}Pi was applied to their roots. The major labeled ester was P-choline, which contained 20% of the total [^{32}P]phosphorus in the xylem exudate from tomato, and 6% from barley under the experimental conditions. The other phosphate ester, which constituted less than 1% of the ^{32}P, is identified in this report as P-ethanolamine. Some acid-soluble organic phosphorus compounds have also been detected in xylem exudates from pumpkin roots (7).

There have been reports that only Pi is present in xylem exudates (9). Exudate collected from passively exuding willow roots, labeled with ^{32}Pi, contained phosphate esters, but xylem exudate collected by vacuum extraction did not contain detectable phosphate esters (13). Similarly when xylem exudate was collected by mild suction from tea plants, organic phosphorus compounds were not detected (16).

The half time for acid hydrolysis of P-choline is 30 to 38 h (8), which is attributed to its very stable structure as an intramolecular ammonium salt or zwitter ion (10). This resistance to hydrolysis has led to erratic results when investigators have attempted to estimate the Pi and ester phosphate content of xylem exudate by conventional acid hydrolysis (7, 9, 18). Therefore, we have used ^{32}P-labeling kinetics and enzymic hydrolysis to measure the P-choline pools. The purposes of these investigations were to determine the relationship between the amount of Pi and the phosphate esters, P-choline and P-ethanolamine, in xylem exudate and to examine the physiological significance of their presence in xylem exudate of tomato plants.

MATERIALS AND METHODS

Reagents. Alkaline phosphatase (EC 3.1.3.1) type VII, P-choline, and P-ethanolamine were from Sigma. [^{32}P]Orthophosphate was from Amersham, and [1,2-^{14}C]choline and [1-^{14}C]ethanolamine were from Research Products. [^{32}P]Choline was synthesized using choline kinase (Sigma) and [γ-^{32}P]ATP (Amersham) and then chromatographically purified. Other reagents were from Mallinckrodt and Sigma.

Plants. Tomato (*Lycopersicon esculentum*) var VF-36 plants were grown in aerated nutrient solution containing 8 mM KNO_3, 8 mM $Ca(NO_3)_2$, 1 mM NH_4NO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 24 μM $NaBO_3$, 6 μM $MnCl_2$, 42 μM $ZnCl_2$, 8 μM Fe-EDTA, 0.05 μM $NaMoO_4$, and 0.16 μM $CuSO_4$. Daylength was 16 h (300 μE m^{-2} s^{-1}) and temperature was maintained at 25°C day and 20°C night. Two plants were kept in each 200-ml polystyrene beaker and supported by circular pieces (2.5 × 6.5 cm) of high density polyurethane. The plants were 20 to 40 d old when used for labeling experiments. Nutrient solutions were changed daily except when plants were grown in very low concentrations of Pi. For experiments examining the effects of Pi concentration, plants were grown in 50- and 100-L containers which were stirred by vigorous aeration. Pi concentration was monitored daily in these containers and KH_2PO_4 was added to maintain the desired concentrations of Pi.

Radiochemical Labeling and Collection of Exudate. The beakers containing the plants were kept in a water bath at 25°C from the time of adding tracer quantities of ^{32}Pi. If the root tempera-

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ture was reduced below 20°C, exudation was slowed or stopped entirely. Plants were cut below the cotyledonary node with a razor blade and allowed to exude for 3 min. The stumps of the stems were blotted dry, and collection of exudate with a syringe was started. The exudate fluid was put into acid-washed graduated centrifuge tubes, which were standing in an ice bath. In some experiments, labeled xylem exudate was also directly transferred onto the origin of 2.5- × 46-cm strips of washed Whatman 3 chromatographic paper. The collection period was 30 min unless otherwise noted. [1,2-^{14}C]Choline and [1-^{14}C]ethanolamine with specific activities varying from 0.2 to 0.4 Ci mol^{-1} were added to the nutrient solutions for a final concentration of 100 μM unless otherwise noted.

Long term ^{32}Pi labeling experiments were done by placing plants in a 20-L glass tank which contained 100 μM Pi in the standard nutrient solution at 25°C. The specific activity of the ^{32}Pi in the tank varied from 2 × 10^{-5} to 6.0 Ci mol^{-1} as indicated in figure legends. This culture size allowed a number of plants to be labeled simultaneously with [^{32}P] at the same concentration and specific activity without severe phosphate depletion.

Determination of Inorganic and Organic Phosphate. The ester-P content of xylem exudate was determined by measuring the difference in Pi content of samples of xylem exudate before and after treatment with alkaline phosphatase. Because acid phosphatase did not hydrolyze P-choline at a significant rate, an alkaline phosphatase had to be used. Aliquots of 25 μl exudate were added to 0.225 ml of buffer containing 50 mM $NaHCO_3$ at pH 10.4, 1 mM $MgCl_2$, 0.1 mM $ZnSO_4$, and 0.5 μg of alkaline phosphatase (1 unit) in acid-washed tubes. After incubating the samples for 16 to 18 h, 0.75 ml of 6% TCA was added, and Pi was determined. Pi content of the xylem exudate was determined by mixing 25 μl of xylem exudate with 0.225 ml of 6% TCA. The increase in Pi from the phosphatase treatment was attributed to the organic phosphate esters. Phosphorus determinations (3) were performed by adding an equal volume of a freshly made mixture of 0.5% $(NH_4)_6Mo_4O_{24}$, 1.2 N H_2SO_4, and 2% ascorbic acid to the samples, as well as standards containing 0 to 100 nmol of phosphate. The color was developed for 90 min at 37°C, and absorbance was read at 820 nm. This method of enzymic hydrolysis quantitatively hydrolyzed 0 to 200 μmol P-choline or P-ethanolamine added to tomato xylem exudate. These phosphate esters were not hydrolyzed by the TCA treatment or during the phosphate determinations.

Data have been expressed on the basis of the fresh weight of the root. When only radioactivity of ^{32}P or ^{14}C was measured, the data were expressed as cpm per min of collection per g fresh weight of root. Amounts of Pi and P-esters, as nmol min^{-1} (fresh weight root)$^{-1}$, were calculated from their concentrations in xylem exudate using the equation (nmol ml^{-1}) × (ml exudate collected min^{-1}) ÷ (g fresh weight root).

[^{32}P]Phosphate and [^{32}P]Choline Distribution in the Leaves. Tomato plants 38 d old with eight to nine leaves were cut at the cotyledonary node and placed in 13- × 100-mm test tubes containing a 1:5 dilution of xylem exudate which had been collected from other plants. They were illuminated with a photoflood lamp at a light intensity of 300 μE m^{-2} s^{-1} and kept at about 25°C by blowing a stream of water-saturated air over the leaves. A 20-min pulse of either ^{32}Pi or [^{32}P]choline was added to the tubes. Both compounds were added at a concentration of 1 mM and a specific activity of 1.9 × 10^{-5} Ci mol^{-1}. This was followed by a 20-min chase with the 1:5 dilution of xylem exudate. The leaves were removed and radioactivity was counted in 5- × 0.5-cm Petri plates by a Geiger-Muller tube.

Extraction of Plant Material. Plant material was suspended sequentially in isopropanol at 50°C for 10 min, in $CHCl_3$:methanol (2:1) for 30 min, and then in 20 mM $CaCl_2$ in water at 100°C. This procedure extracted both the water- and lipid-soluble forms of choline and ethanolamine. Degradation products from P-lipids were not seen on the chromatograms.

Separation of Labeled Compounds. Compounds were separated by two dimensional chromatography on paper or cellulose TLC plates developed with water-saturated glass-distilled phenol in the first direction and butanol:propionic acid:H_2O (2:1:1.3) in the second direction. Labeled material was located by autoradiograms. Ethanolamine and P-ethanolamine were located with a ninhydrin spray; choline and P-choline were located with Dragendorf's reagent; the R_F values with H_2O-saturated phenol were 0.9 for P-choline, 0.3 for P-ethanolamine, and 0.1 for Pi. On 0.25-mm TLC plates developed with butanol:propionic acid:H_2O (2:1:1.3), the R_F values were 0.42 for P-choline, 0.35 for P-ethanolamine, 0.63 for choline, and 0.57 for ethanolamine. Pi was detected by an acid molybdate spray test. Pi was also separated from phosphate esters by chromatographing 50 μl of labeled exudate on a 0.5- × 1.0-cm column of Dow-1-HCOOH (400 mesh). P-choline and P-ethanolamine were eluted with 2 ml of 0.01 N HCl, and Pi with 2 ml of 1 N HCl.

RESULTS

Xylem exudate from tomato plants contained only three ^{32}P-labeled compounds and they could be separated either by paper chromatography, TLC plates, or Dowex-1-HCOOH column chromatography, as previously reported (10, 18). The distribution of ^{32}P and the amount of these three compounds varied as described in subsequent sections, but Pi was always the major component. P-Choline contained 2 to 30% of the ^{32}P and P-ethanolamine was present at about one-tenth the concentration of P-choline.

Identification of P-Ethanolamine in Xylem Exudate. The identification of P-choline in xylem sap was confirmed as previously published (10). The other P-ester originally described in barley and tomato xylem exudates as unknown 2 (10, 18) was the only other labeled P-ester in tomato xylem exudates after feeding ^{32}Pi. Large volumes (ml) of labeled xylem exudate were added to a Dow-1-formate column (1.5 × 50 cm), which had been washed with 20 ml of 5 mM $NaBO_3$ at pH 8.5. The column was developed with a 0 to 0.4 M ammonium formate gradient in the same buffer. Pi was not eluted but P-choline was eluted with 0.1 M ammonium formate. The other ^{32}P-labeled material, which was

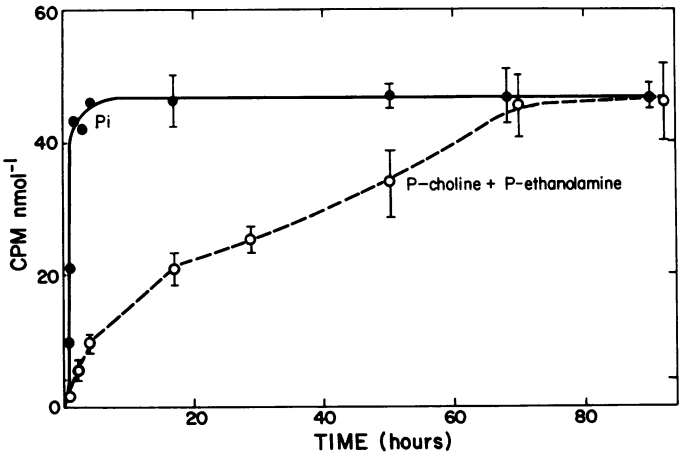

FIG. 1. Specific activities of phosphate and P-choline plus P-ethanolamine in xylem exudate of tomato plants after adding ^{32}Pi to the nutrient. The nutrient solution had 100 μM ^{32}Pi with 47 cpm nmol^{-1}. The labeled P-esters were separated by Dow-1-formate columns, and the quantities were determined as phosphate by the molybdate method after hydrolysis with alkaline phosphatase. Each time point represents a different set of plants in triplicate that were cut off below the cotyledonary node before collecting exudate fluid for 30 min.

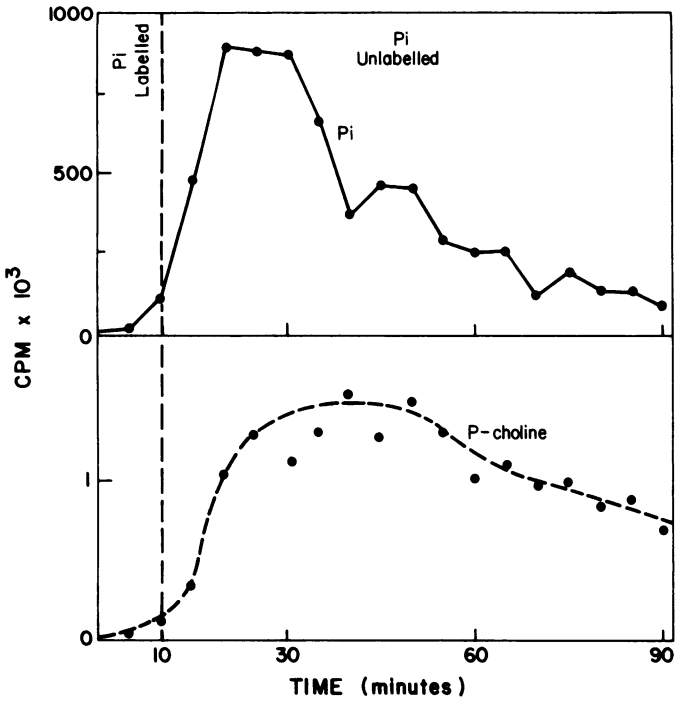

FIG. 2. ^{32}P in phosphate and P-choline of xylem exudate after a 10-min pulse with ^{32}Pi and an 80-min chase with unlabeled phosphate. Two plants in 200 ml of nutrient with 100 μM Pi were labeled for each time point. P-choline was separated from Pi by paper chromatography with 80% phenol-water.

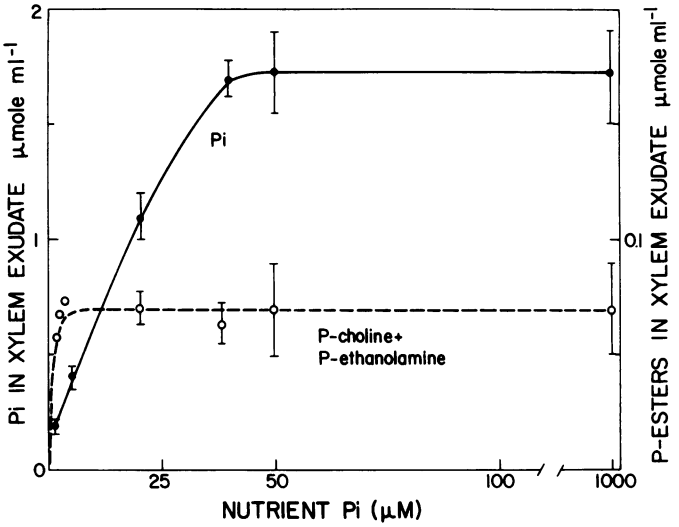

FIG. 3. Concentrations of Pi and P-esters in xylem exudate as a function of Pi concentration in the nutrient solution. The plants were grown at the indicated Pi concentrations for 20 d prior to the experiment, the exudate was collected for 30 min, and phosphate compounds were analyzed quantitatively as described in "Materials and Methods."

eluted by 0.3 M ammonium formate, co-chromatographed with authentic P-ethanolamine on cellulose TLC plates in three solvent systems (water-saturated phenol, butanol:propionic acid:water [2:1:1.3], and methanol:ammonia:water [6:1:3]). The labeled compound also co-migrated with authentic P-ethanolamine in high voltage electrophoresis (260 mamps for 10 min) with 0.1 M $NaBO_3$ as an electrolyte. In addition, this P-ester was ^{14}C-labeled in the xylem exudate within 0.5 h after feeding [^{14}C] ethanolamine to tomato roots. After the ^{14}C-labeled compound was hydrolyzed by alkaline phosphatase, it co-chromatographed

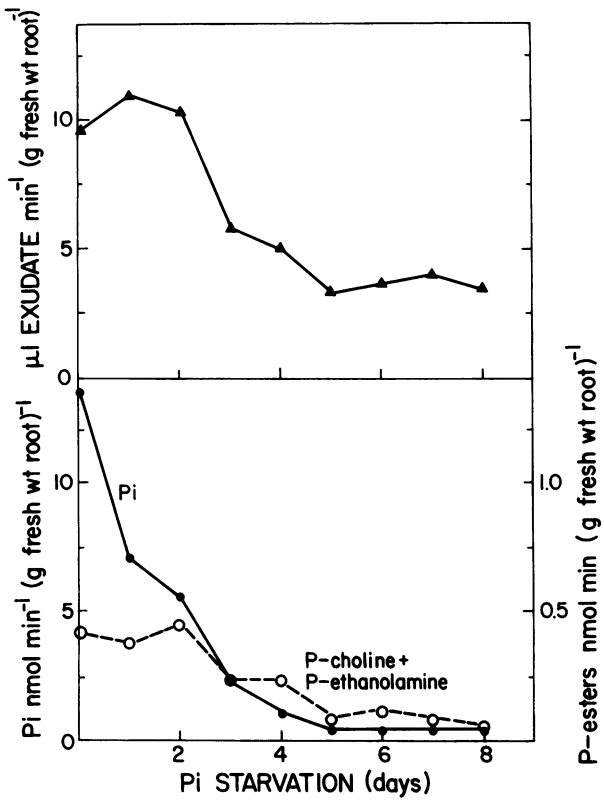

FIG. 4. Effect of phosphate starvation on the amount of xylem exudate and the amounts of phosphate and P-esters in xylem exudate. The plants had been grown in 1 mM Pi for 41 d and then transferred to a minus Pi nutrient that was changed daily. (●——●), Pi; (0- - -0), P-esters. Each data point represents the composition of xylem sap from plants cut every 24 h. Sap was collected for 30 min.

with ethanolamine in the above solvents.

^{32}P Labeling Kinetics of Phosphate, P-Choline, and P-Ethanolamine. When ^{32}Pi was added to the nutrient solution of whole tomato plants, ^{32}P in Pi of the xylem exudate collected from these plants reached, within 4 h, the same specific activity as that in the nutrient solution (Fig. 1). The phosphate moiety of P-choline and P-ethanolamine in the xylem exudate however, did not attain the specific activity of the ^{32}Pi in the nutrient solution until 72 to 96 h later. After 96 h, at the end of the experiment, 87% of the label in the P-ester fraction was in P-choline. The rapid labeling of Pi in the xylem exudate suggests that Pi was loaded into the xylem from a small pool which exchanged slowly with the bulk of the Pi in the root. In contrast, the P-esters were labeled from a slowly exchanging pool of Pi, which can be considered to be the metabolic pool. P-Choline and P-ethanolamine were labeled at the same rate and thus were probably in equilibrium with the same source of phosphate. Inasmuch as the plants had been grown in a normal nutrient solution, with 1 mM phosphate, their total Pi content was large. The anatomical location of the metabolic pool is unknown, but it has previously been described as different from the root pool of Pi (4).

Due to the different time scales for labeling Pi and the P-esters, the ^{32}P in the xylem exudate was initially nearly all Pi. With time, the percentage of ^{32}P in P-choline increased to a maximum value of about 20% under these experimental conditions.

Tomato plants were given a 10-min pulse of ^{32}Pi in the nutrient solution, then decapitated, and the nutrient was replaced with unlabeled nutrient solution. Pi in the xylem exudate was maximally labeled within the next 10 min (Fig. 2). Twenty min after the pulse, label in Pi began to decline as unlabeled Pi entered the

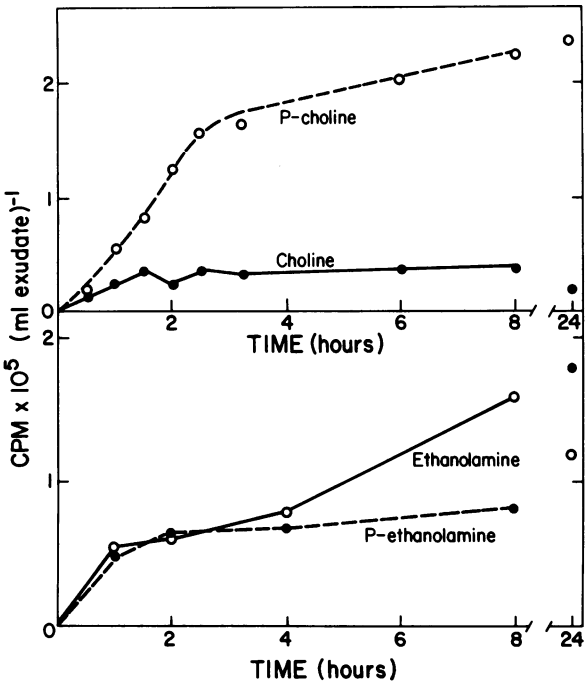

FIG. 5. Labeled P-choline and P-ethanolamine in xylem exudate after adding [^{14}C]choline and [^{14}C]ethanolamine to the nutrient. At zero time, [1,2-^{14}C]choline (991 cpm nmol^{-1}) or [1-^{14}C]ethanolamine (598 cpm nmol^{-1}) was added to the nutrient to form 100 μM solutions, and at subsequent times the stems were cut off and xylem exudate collected for 30 min. Compounds in the exudate were separated by TLC developed with butanol:propionic acid:water (2:1:1.3).

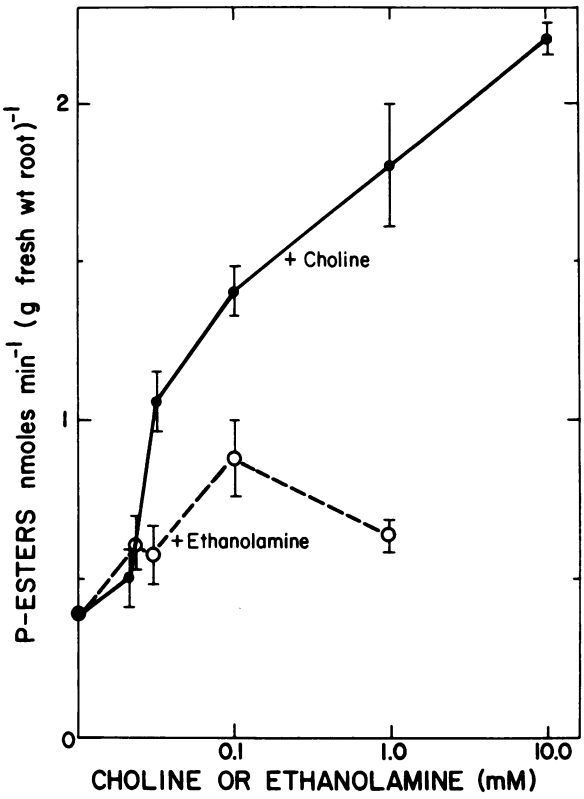

FIG. 6. Effect of exogenous choline and ethanolamine on the flux of P-esters into xylem exudate. The compounds at indicated concentrations in the nutrient solution were present for 16 h before the plants were decapitated and xylem exudate collected for 30 min. The plants used for this experiment were 40 d old.

xylem sap. This labeling pattern is similar to that reported in the leaves of intact barley seedlings, after their roots were given a 5-min pulse of ^{32}Pi, followed by a 6-h chase (4). P-Choline in the xylem exudate was maximally labeled about 40 min after the ^{32}Pi pulse, and then the label in it declined only slowly (Fig. 2). In this experiment, 0.1% of the total label was in P-choline 30 min after the beginning of the ^{32}Pi pulse, but the percentage increased to 1 to 2% after 80 min of chase. These results are consistent with the hypothesis that Pi in the xylem sap comes mainly from a rapidly exchanging root pool, although the slowly exchanging metabolic pool of Pi also contributes. The slower labeling and slower turnover of label in P-choline indicates that its phosphate moiety comes from the metabolic pool of Pi.

Effects of Nutrient Phosphate Concentration. The amount of Pi in xylem exudate of tomato plants increased with increasing Pi concentration of the nutrient solution up to 40 μM (Fig. 3), which is comparable to Pi transport in wheat (5). The flux of the P-choline and P-ethanolamine into xylem exudate was maximum with 1 to 2 μM Pi in the nutrient solution. Plants grown in 2 μM Pi were as large as those grown in 40 μM Pi, but had foliage which was darker green, which indicated phosphate deficiency. Plants grown in Pi concentrations below 1 μM were small and had purple green foliage, severe symptoms of Pi deficiency. At 2 μM Pi, P-esters represented approximately 30% of the phosphate in the xylem exudate of the tomato. This value is similar to the percentage of total phosphate reported in the organic form in xylem exudates collected from field-grown corn (14), and 2 μM Pi is in the range expected in most U.S. soils (2). In nutrient solution containing 1 mM Pi, the P-esters represented only 1 to 2% of the total phosphate in xylem exudate due to the large amount of Pi but the amount of P-ester was the same as at lower nutrient Pi levels. We have examined xylem exudate collected from field-grown tomato plants in Michigan and have found a

Table I. *Distribution of Radioactivity in Tomato Plants after Feeding [1,2-^{14}C]Choline or [1-^{14}C]Ethanolamine*

The compounds were added to a concentration of 0.1 mM in the nutrient solution after which the plants were decapitated every 2 h and the xylem exudate was collected for 30 min. Then all of the plant was assayed for distribution of the ^{14}C label as presented in the lower part of the table. The specific activity of [1,2-^{14}C]choline was 598 cpm nmol^{-1} and [1-^{14}C]ethanolamine was 991 cpm nmol^{-1}. Choline and ethanolamine from the xylem sap were separated from their P-esters by TLC developed in butanol:propionic acid:water (2:1:1.3). The mean fresh weights were 0.111 ± 0.015 g (roots), 0.324 ± 0.022 (leaves), and 0.097 ± 0.022 (stems).

Time	Labeled Compounds in Xylem Exudate			
	[1,2-^{14}C]Choline added		[1-^{14}C]Ethanolamine added	
	P-Choline	Choline	P-Ethanolamine	Ethanolamine
h	*cpm ^{14}C min^{-1} (g fresh weight root)$^{-1}$*			
2	5,810	590	250	270
4	3,810	800	190	160
6	4,300	730	240	260

Time	Distribution of ^{14}C into Plant Tissue					
	[1,2-^{14}C]Choline			[1-^{14}C]Ethanolamine		
	Leaves	Stems	Roots	Leaves	Stems	Roots
h	*cpm min^{-1} (g fresh weight root)$^{-1}$*					
2	240	1,790	8,850	186	150	295
4	2,320	2,410	12,620	188	151	330
6	3,900	2,260	12,220	143	362	524

Table II. *Distribution of ^{32}Pi and [^{32}P]Choline among the Leaves of Excised Tomato Shoots*

A 20-min treatment with the labeled compounds was followed by a 20-min chase with the corresponding unlabeled compound. The values represent means and the SD from two experiments, each of which had two replications per treatment. The leaves were numbered from the bottom of the shoot.

Leaf No.	Added Radioactivity in Compounds	
	^{32}Pi	[^{32}P]Choli
	%	
1	3.2 ± 1.0	3.5 ± 1.9
2	10.9 ± 4.6	9.0 ± 4.4
3	13.0 ± 3.5	12.5 ± 7.7
4	23.9 ± 11	16.9 ± 1.5
5	21.8 ± 5.3	25.2 ± 7.4
6	12.0 ± 3.4	13.7 ± 6.3
7	5.6 ± 3.3	4.9 ± 3.0
8	1.1 ± 0.4	2.2 ± 0.5
Meristem	0.4 ± 0.1	0.9 ± 0.4
Stems	8.3 ± 0.8	11.2 ± 3.8

mean percentage of 17% of the total phosphate as P-esters (11).

Effects of Phosphate Starvation. When tomato plants grown in 1 mM Pi were placed in nutrient solution without Pi, the concentration of Pi in xylem exudate decreased from 1400 to 170 μM in 7 d (Fig. 4). The exudation rate of the plants declined at least 2-fold after the 3rd d of Pi stress, thereby accounting in part for the decline in the total amount of Pi and P-esters collected per unit of time. The concentration of the P-esters, P-choline, and P-ethanolamine in the xylem exudate remained constant during this period, at 35 μM, although their flux into the xylem declined due to less exudation. While the amount of Pi in the xylem exudate was greatly affected by the Pi concentration of the nutrient solution and the root pool, no effect of short term Pi availability on the concentration of the P-esters was apparent. When plants were held in minus Pi nutrient for longer times (14, 28, or 35 d), they failed to exude.

Effect of Adding Choline and Ethanolamine on P-Esters in Xylem Exudate. When [1,2-^{14}C]choline was added to the nutrient solution and xylem exudate was collected 8 h later, 90% of the ^{14}C label was in P-choline and the rest was in choline (Fig. 5). When [1-^{14}C]ethanolamine was used for the same time interval, less than 50% of the label was in P-ethanolamine and the rest was in ethanolamine. The ^{14}C specific activity of the labeled P-esters in the xylem exudate approached that of the added compound in the nutrient within 2 h. In these experiments, an increase in total P-choline and P-ethanolamine was observed in the xylem exudate (data not shown) when the labeled choline or ethanolamine were added to the nutrient solution. We hypothesized that normal endogenous pools of choline and ethanolamine were apparently limiting the amount of P-esters in the xylem exudate. To test this idea further, various concentrations of choline or ethanolamine were added to the nutrient solution and the compounds in the xylem exudate were measured 24 h later. The flux of Pi was unaffected by these treatments (data not shown). The amount of total P-esters, P-choline plus P-ethanolamine, was significantly increased by 50 μM choline or 100 μM ethanolamine (Fig. 6). Addition of higher concentrations of ethanolamine did not further increase the P-esters in the exudate. With the addition of up to 10 mM choline in the nutrient, the P-esters, mainly P-choline, continued to increase in the xylem exudate to over 2 nmol min^{-1} (g fresh weight root)$^{-1}$. Since tomato roots can form up to 10 nmol min^{-1} (g fresh weight root)$^{-1}$ of either P-choline or P-ethanolamine (11) by kinase activity, it seems unlikely that ATP concentration limited the P-ester flux into xylem exudate of these plants. The large increases in P-esters in the exudate would appear to be the result of increased availability of choline or ethanolamine, which would normally be limiting. The lower flux of P-esters observed with added ethanolamine may be the combined result of slow conversion of exogenous ethanolamine to choline in tomato roots (11) and the naturally lower concentration of P-ethanolamine in xylem exudate in comparison to P-choline.

Labeling of Whole Plants. Four-week-old, intact tomato plants were given 100 μM of either [1,2-^{14}C]choline or [1-^{14}C]ethanolamine in the nutrient solution. Different plants were cut off every 2 h and xylem exudates were collected for 30 min. The rates of accumulation of radioactivity in the leaves, stems, and roots as well as the flux of P-choline and choline or P-ethanolamine and ethanolamine into the xylem exudate are presented in Table I. After 2 h, the flux of P-choline into the xylem exudate was nearly constant at 5.4 ± 0.9 nmol min^{-1} (g fresh weight root)$^{-1}$. This high rate was approximately 10-fold greater than that of controls without choline added to the nutrient solution. The mean accumulation rate of choline and P-choline into the leaves during the period of 2 to 6 h after adding ^{14}C-labeled choline to the roots was 5.5 ± 1.0 nmol min^{-1} (g fresh weight root)$^{-1}$. The rate of accumulation of label into the leaves during the time period 2 to

Table III. *Estimated Contribution of P-Choline from the Xylem Stream to Total Phosphatidyl Choline Synthesis in Leaves of Tomato Plants*

Plant Age	Mean Wet Wt			Calculated Rate of Phosphatidyl-choline, Synthesis[a]	Observed Rate of P-Choline Import[b]	Estimated Leaf Phosphatidylcholine Synthesized from Transported P-Choline
	Roots	Leaves	Mean Leaf Growth Rate/Unit Time			
d		*g*		*nmol min^{-1} (g leaf wet wt)$^{-1}$*		%
12	0.0056	0.006	0.02	0.003		
20	0.055	0.182	1.0	0.017	0.12	700
25	0.095	0.305	1.0	0.17	0.13	95
31	0.785	1.95	11.4	0.19	0.17	86
35	1.66	4.04	21.8	0.36	0.16	39
40	2.44	7.08	25.3	0.42	0.14	33
45	2.46	8.83	14.6	0.24	0.11	46
50	7.53	11.60	23.0	0.38	0.26	68

[a] Required rate based on 1.0 μmol phosphatidylcholine (g fresh wt leaf)$^{-1}$.

[b] Based on 0.4 nmol P-choline (g fresh wt root)$^{-1}$ min^{-1} movement into xylem exudate.

6 h after feeding ^{14}C-labeled ethanolamine was 0.76 ± 0.18 nmol min^{-1} (g fresh weight root)$^{-1}$. The data indicate that the flux rates of P-choline and P-ethanolamine into xylem exudate of decapitated plants were approximately the same as the rate of accumulation of label from these P-esters into leaves of whole plants. This is contrary to the increases observed in Pi flux with increased transpiration (6).

Excised tomato shoots of 30-d-old plants were given a 20-min pulse of either ^{32}Pi or [^{32}P]choline, followed by a 20-min chase with the unlabeled compounds. The distribution of radioactivity among the leaves was not significantly different for Pi and P-choline (Table II). It appears that both compounds follow the xylem stream into the leaves without significant xylem to phloem transfer as is the case with the amino acids, valine and asparagine, in the xylem of legumes (12).

Contribution of P-Choline in the Xylem Stream to Phosphatidylcholine Synthesis in the Leaves. Tomato leaves incorporate P-choline labeled with ^{32}P or ^{14}C, which had arrived via the xylem, into phosphatidylcholine with little hydrolysis to Pi and choline (11). The flux of P-choline into xylem exudate was compared with the rate of labeled phosphatidylcholine synthesis in leaves of intact plants (Table III). The assumption was made that P-choline flux in the xylem stream of whole plants was the same as in xylem exudate of decapitated plants. Data in Table I support the validity of this assumption. This flux had a mean value of 0.4 nmol P-choline min^{-1} (g fresh weight root)$^{-1}$, which is the control value in Figures 4 and 6. The total pool size of phosphatidylcholine in tomato leaves was approximately 1.0 μmol (g fresh weight leaf)$^{-1}$ (15). It can be assumed also from data in Table II that P-choline movement into leaves followed the xylem stream with minimal redistribution by the phloem tissue (1).

P-Choline import was estimated to exceed or equal the rate of phosphatidylcholine synthesis in leaves of plants younger than 25 d of age. The xylem exudate that could be collected from decapitated older plants may greatly underestimate the true xylem flow, but it still contained about half the quantity of P-choline needed for phosphatidylcholine synthesis in the leaves. Although the calculations in Table III are only approximate and tend to oversimplify the complicated process of plant growth, they suggest that the P-choline synthesized in the roots and carried by the xylem stream makes a contribution to phospholipid synthesis in tomato leaves.

DISCUSSION

In 1956, P-choline was identified as a component of xylem exudate (10, 18). It exists as an intramolecular neutral ammonium salt, which the many other phosphate esters cannot form, and this could allow P-choline to cross membranes. Whether P-choline transport is an active or passive process is not known. Several reasons may account for why P-choline has usually not been detected and studied in plant metabolism except in a few reports with ^{32}Pi; P-choline is resistant to strong acid hydrolysis, it is not rapidly hydrolyzed by acid phosphatases, it is not readily detected by phosphate or ninhydrin spray tests, and amounts of it are relatively small compared to the other phosphate esters in leaf tissue. In this paper, a quantitative assay for P-choline has been developed based on its hydrolysis by alkaline phophatase. The presence of considerable amounts of P-choline in the xylem sap had raised the possibility that it was an organic phosphate ester for phosphate and nitrogen transport, as glutamate, glutamine, and allantoin are organic nitrogen compounds for transport of nitrogen (18).

Little correlation between the amount of Pi and P-choline in the xylem sap was observed in this study. The Pi concentration in xylem exudate of tomatoes depended on the nutrient Pi concentration up to 50 mM Pi, while the amount of P-choline in the xylem fluid was independent of nutrient Pi greater than 1 μM (Fig. 3). Pi starvation also reduced Pi in the xylem exudate but had little effect on the amount of P-choline present (Fig. 4). The amount of P-choline and P-ethanolamine in xylem sap were influenced by the choline and ethanolamine pool sizes in the root (Fig. 6). Our data suggest Pi and P-choline transport in xylem exudate were independent processes. However, under field conditions the concentration of Pi may be around 1 μM and could influence the amount of P-choline in the xylem exudate, as was observed with field-grown tomato plants (11).

[^{32}P]Choline was transported to all the leaves in apparent proportion to the xylem transpiration stream. Roots have an active choline kinase that can form 10 nmol P-choline min^{-1} (g fresh wt root)$^{-1}$ (11). Choline kinase activity should be adequate for the maximum flux observed in the xylem exudate of 5 nmol P-choline min^{-1} (g fresh weight root)$^{-1}$. In a previous investigation of the properties of this root kinase, a 10-fold lower level of activity had been reported (17). The properties and location of choline kinase in the root are still unknown. We now propose that P-choline is synthesized in the roots and transported in the xylem fluid to the leaves where it is used for lipid synthesis. The magnitude of this transport indicated that P-choline from the root could be sufficient to account for total phosphatidylcholine synthesis by the CDP-choline pathway in young tomato plants up to 20 d of age (Table III). Thus, the roots provide this important metabolite for phospholipid formation in the leaves. The aerial portions of older plants may synthesize part of their P-choline or they may form phosphatidyl choline via a route not involving free P-choline as an intermediate.

In our earlier work with ^{32}Pi (18), the data were expressed as a percentage of the total ^{32}P in the xylem exudate. Values ranging from a fraction of 1% to 20% were observed. In this paper, the rate of labeling of the xylem Pi after adding ^{32}Pi to the nutrient was very rapid and reached the specific activity of the added Pi within 4 h for 2- to 4-week-old tomato plants (Fig. 1). P-Choline in the xylem sap was labeled more slowly and did not attain the specific activity of the added ^{32}Pi for 76 to 96 h. Then P-choline represents 20 to 30% of the ^{32}Pi in the xylem exudate, but at 4 h it would be only about 1% of the total ^{32}P, even though as much unlabeled P-choline was in the xylem fluid flux. We have expressed therefore most of the data as nmol P-choline. In Pi-deficient plants, up to 30% of the total phosphate in xylem exudate was present in the phosphate esters, P-choline and P-ethanolamine (Fig 4).

The flux of Pi and P-choline was calculated by measuring the volume of exudate collected over a 30-min period and expressing the data relative to the wet weight of the roots (Table III). These analyses could underestimate the flux into the xylem exudate since part of the fluid from the decapitated stem may move back down the phloem before it could be collected. Further, the loss of the top meant that the volume of exudation could be less than true xylem flow as only root pressure without transpiration pull was producing the exudate.

The other P-ester in xylem exudate was identified as P-ethanolamine. In barley and in tomato plants, it was present at about one-tenth of the amount of P-choline. P-Ethanolamine, like P-choline, can also form an internal ammonium salt and thus move across membranes. Its metabolism in the roots and leaves has been considered, along with P-choline, elsewhere (11).

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